Identification of a Receptor Mediating Absorption of Dietary Cholesterol in the Intestine†

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ABSTRACT: Here we show that scavenger receptor class B type I is present in the small-intestine brush border membrane where it facilitates the uptake of dietary cholesterol from either bile salt micelles or phospholipid vesicles. This receptor can also function as a port for several additional classes of lipids, including cholesteryl esters, triacylglycerols, and phospholipids. It is the first receptor demonstrated to be involved in the absorption of dietary lipids in the intestine. In liver and steroidogenic tissues, the physiological ligand of this receptor is high-density lipoprotein. We show that binding of high-density lipoprotein and apolipoprotein A-I to the brush border membrane-resident receptor inhibits uptake of cholesterol (sterol) into the brush border membrane from lipid donor particles. This finding lends further support to the conclusion that scavenger receptor BI catalyzes intestinal cholesterol uptake. Our findings suggest new therapeutic approaches for limiting the absorption of dietary cholesterol and reducing hypercholesterolemia and the risk of atherosclerosis.

Fats and sterols ingested in the diet leave the stomach and enter the small intestine as emulsion droplets. The following three processes then occur to promote absorption of the water-insoluble lipid molecules into intestinal epithelial cells (enterocytes) (*1*−*4*). First, hydrolysis of triacylglycerol and phospholipid (PL) molecules by pancreatic lipases gives rise to more polar molecules such as free fatty acids. Second, the lipids and products of lipid hydrolysis are dispersed in the lumen of the small intestine by bile salts, yielding as the main natural lipid carriers bile salt-containing micelles and small unilamellar vesicles (SUV)[1] (*3*). These small lipid particles transport the water-insoluble lipids via Brownian diffusion to the surface of the small intestine and raise the concentration of fatty acid, monoacylglycerol, and cholesterol molecules near the brush border membrane (BBM) of enterocytes (*1*). Third, these lipid molecules are transferred into the BBM, from where they are released into the cytosol of the enterocytes.

The mechanism by which lipid molecules are transferred from the lipid carrier to the BBM is still a matter of debate. The prevailing view documented in text books is that the process of lipid transfer is simple passive diffusion. This mechanism involves the desorption of the lipid molecule from the carrier particle, diffusion of the lipid through the aqueous phase, and incorporation of the lipid molecule into the lipid bilayer of the BBM. However, for water-insoluble lipids with very low monomer solubility ($\leq 10^{-8}$ M) such as cholesterol and cholesteryl esters, this mechanism is inefficient. Indeed, we have presented kinetic and other evidence showing that the transfer of free and esterified cholesterol to the BBM is a facilitated process (*5*−*8*). It involves collision-induced transfer of the lipid molecule from the lipid carrier particle to the BBM, and this transfer is protein-mediated. On the other hand, if the monomeric solubility of a lipid in water is relatively high like for monoacylglycerols (*9*) ($\geq 10^{-6}$ M), the simple diffusion mechanism (*10*) is effective and contributes significantly to the overall absorption.

Up to now, the role of a protein-mediated pathway of lipid absorption has been poorly understood because no receptor has been identified. Here we show that a previously identified

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[1] Abbreviations: apo, apolipoprotein; BCA, bicinchoninic acid; BBM, brush border membrane(s); BBMV, brush border membrane vesicle(s); bp, base pair(s); CLA-1, human scavenger receptor class B, type 1 [CD36 and lysosomal integral, membrane protein II (LIMP II)-analogous-1 protein]; HDL, high-density lipoprotein; HPLC, high-pressure liquid chromatography; PAGE, polyacrylamide gel electrophoresis; PC, phosphatidylcholine; PVDF, poly(vinylidene difluoride); SDS, sodium dodecyl sulfate; SR-BI, scavenger receptor class B, type I; SUV, small unilamellar vesicle(s).

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membrane protein, termed scavenger receptor (SR) BI[2] (*11*, *12*), is located in the BBM of enterocytes and that this receptor catalyzes the uptake of dietary sterols and other classes of lipids.

EXPERIMENTAL PROCEDURES

Materials

Egg phosphatidylcholine (PC), egg phosphatidic acid, and dimyristoyl PC were purchased from Lipid Products (South Nutfield, U.K.), trioleoylglycerol and cholesteryl oleate from Sigma (Buchs, Switzerland), [4-^{14}C]cholesterol (~50 Ci/mol), [1α,2α(N)-^{3}H]cholesterol (47 Ci/mmol), [1α,2α(N)-^{3}H]cholesteryl oleyl ether (37 Ci/mmol), and [*carboxyl*-^{14}C]trioleoylglycerol (50−80 Ci/mol) from Amersham, 1,2-diheptanoyl PC and dipalmitoyl PC from Avanti Polar Lipids (Alabaster, AL), a mixture of glycosidases (endoglycosidase F and *N*-glycosidase F) and a 5′/3′ RACE kit from Boehringer (Mannheim, Germany), and a human intestinal λgt11 5′ Stretch cDNA library was obtained from Clontech (Heidelberg, Germany). Rat SR-BI cDNA was kindly provided by B. Carlsson (Sahlgrenska University Hospital, Gothenburg, Sweden). Peptides of 18 amino acids, $18A_D$ (DWLKAFYDKVAEKLKEAF) consisting of D-amino acids, and 20 amino acids, 20P (VSQPGLAAGPDRPPSPYTPL), were synthesized by Genosys (Cambridge, U.K.). Their purity was >95% as assessed by HPLC, and the molecular masses determined by mass spectrometry were consistent with the calculated values.

Methods

Published methods were used for the preparation of mixed bile salt micelles and SUV (*5*−*8*), BBMV from 1.5 m of rabbit duodenum and jejunum (*7*, *13*), the reconstitution of BBMV (*8*), the digestion of BBMV by papain and proteinase K (*14*), the deglycosylation of BBMV proteins by digestion with glycosidases following the Boehringer instruction manual, the preparation of human HDL and apolipoprotein (apo) A-I (*15*), ^{14}C-labeled apo A-I (1 μCi/mg of protein) (*16*), the postnuclear supernatant of murine Y1-BS1 cell lysate (*17*), lipid uptake measurements using BBMV (*5*−*8*) and Caco-2 cells (*18*), immunoelectron microscopy (*19*), SDS−PAGE (*20*), and the determination of phospholipid (*21*) and protein concentrations (BCA method of Pierce Chemical Co.).

Expression of the Luminal Part of Human SR-BI (Lum CLA-1) in Escherichia coli. An 1141 bp fragment of human SR-BI was cloned into the expression vector pET 23a (Novagen, Madison, WI), and the resulting construct with a His tag at the C terminus was transformed into *E. coli* BL21-(DE3) cells and expressed. The recombinant protein containing amino acids L41−A421 together with 24 N-terminal and 4 C-terminal vector-derived amino acids and a $(His)_6$ tail at the C terminus was present in inclusion bodies. It was purified by disrupting the cells [1.5 g wet weight *E. coli* pellet suspended in 7.5 mL of 20 mM sodium phosphate buffer (pH 7.4) containing 0.5 M NaCl] by short bursts of sonication (Branson sonifier), centrifuging the suspension at 20000*g* for 15 min at 4 °C, and washing the pellet obtained twice with the same buffer. The pellet was incubated with 5 mL of the same buffer containing 6 M guanidine hydrochloride at 4 °C overnight. After centrifugation at 39000*g* for 20 min, the protein in the supernatant was purified on a Ni^{2+} column according to the recommendations of the manufacturer (Novagen); it gave a single band on SDS−PAGE (9.5%) with an apparent molecular mass of 45 kDa, in good agreement with the molecular mass of 46.8 kDa calculated from the amino acid composition. The yield from 1.5 g wet weight of *E. coli* cells was about 25 mg of Lum CLA-1 protein.

Production and Purification of Polyclonal Antibodies Raised against Human SR-BI. Polyclonal antibodies against residues 230−328 of human SR-BI (pAb 1336) and the C-terminal region of the SR-BI isoform (pAbI2) (*22*) were raised in New Zealand white rabbits as described in ref *23*. In the latter case, the peptide 20P coupled to key hole limpet hemocyanin was used as the antigen.

IgG was isolated by protein G Sepharose (Pharmacia) fast flow column chromatography (*24*) of the antiserum. It was further purified by immunoprecipitation or affinity purification. For immunoprecipitation, equal volumes (0.75 mL) of IgG (1.2 mg/mL) and Lum CLA-1 (0.67 mg/mL) in 50 mM sodium phosphate buffer (pH 7.7) were incubated overnight at 4 °C on a turning wheel. The immunoprecipitate thus formed was washed twice via resuspension and centrifugation at 5000*g* for 5 min. IgG was liberated from the immunoprecipitate by incubating the pellet with 50 mM glycine (pH 2) for 3 min at 25 °C and neutralizing with 1 M Tris (pH 7.5). For affinity purification of antisera or anti-human SR-BI IgG, purified Lum CLA-1 was resolved on SDS−PAGE (9.5%) and blotted onto a PVDF membrane; five PVDF strips containing 40 μg of protein each were incubated with either antiserum or IgG at 4 °C on a turning wheel overnight, and bound antibodies were eluted as described above.

Northern and Immunoblot Analysis. Surgical specimens of human liver and small intestine were obtained from the University Hospital Zurich, and total RNA was extracted from liver homogenate and duodenal enterocytes by the acid guanidine thiocyanate method (*25*). Full-length human SR-BI cloned into the expression vector pcEXV-3 (*26*) was excised with *Eco*RI; the two fragments thus obtained were subjected to random primer labeling (Promega, Madison, WI) and used as probes in Northern analysis.

For immunoblot analysis, membrane proteins were size-fractionated on SDS−PAGE (9.5%) under reducing conditions, transferred to a PVDF membrane, and immunoblotted with polyclonal anti-human SR-BI antibodies. As the secondary antibody, monoclonal anti-rabbit IgG conjugated to alkaline phosphatase (Sigma, St. Louis, MO) was used. Proteins were made visible either by the tetrazolium method (*27*) or by chemiluminescence detection (Immun-star from Bio-Rad, Glattbrugg, Switzerland).

Binding of Apo A-I to Rabbit Small-Intestine BBMV. The extent of binding of apo A-I to BBMV was determined under the conditions of lipid uptake measurements. BBMV (2 mg of protein/mL) and donor vesicles (egg PC vesicles containing 1 mol % [^{3}H]cholesterol, with a total lipid concentration of 0.05 mg/mL) both dispersed in 0.05 M Tris (pH 7.4), 0.15 M NaCl, and 0.02% NaN_3 were incubated with increasing

[2] The human scavenger receptor BI was first identified as cDNA of unknown function and abbreviated CLA-1 for CD36 and lysosomal integral membrane protein II (LIMP II)-analogous-1 protein.

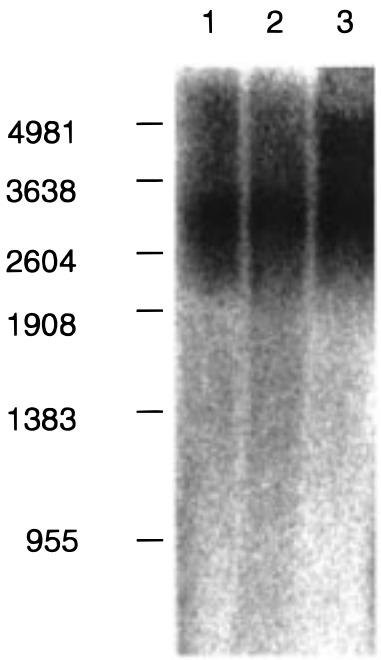

FIGURE 1: Northern blot analysis of SR-BI mRNA expression in various tissues: lane 1, human duodenal enterocytes prepared according to the methods described in ref *19*; lane 2, human liver; and lane 3, differentiated Caco-2 cells. The nucleotide size in bp is given on the left. Equal amounts (6 μg) of total RNA based on the intensity of the 18S and 28S rRNA bands were loaded and run on an agarose gel, blotted onto nitrocellulose, and probed with human SR-BI cDNA (see Methods).

amounts of radiolabeled apo A-I at room temperature for 20 min. BBMV were separated from unbound apo A-I by centrifugation at 115000*g* for 2 min in a Beckman airfuge, and the amount of radiolabeled apo A-I present in the supernatant was determined.

RESULTS

A human intestinal cDNA library was screened with full-length rat SR-BI cDNA using standard protocols. Two independent clones differing in the 3′ region were isolated, the difference being a 129 bp deletion resulting from alternative splicing occurring at the splice donor site of intron 11 (*22*, *28*). Using mRNA isolated from human enterocytes and employing a 5′ RACE strategy yielded clones identical with the N-terminal long form, indicating that the isoform with the N-terminal deletion described in ref *12* is not present in enterocytes. The nucleotide sequence of all clones analyzed was identical to that of human SR-BI (*12*).

Northern analysis of total RNA indicated that the message of SR-BI is present in human enterocytes. Figure 1 shows that the 2.8 kb SR-BI mRNA (*12*, *29*) is expressed not only in liver but also in enterocytes and differentiated Caco-2 cells.[3] Furthermore, the bands obtained with human SR-BI mRNA from these different tissues had similar intensities. Immunoblotting provides further evidence of the localization of SR-BI (Figure 2). Immunoblot analysis of rabbit BBMV and differentiated Caco-2 cells using affinity-purified rabbit anti-human SR-BI IgG (pAb 1336) revealed a predominant

[3] Caco-2 cells are a human intestinal cell line derived from a primary colon adenocarcinoma tumor. Upon reaching confluence, the cells form a polarized monolayer with an apical brush border membrane morphologically comparable to that of the small intestine.

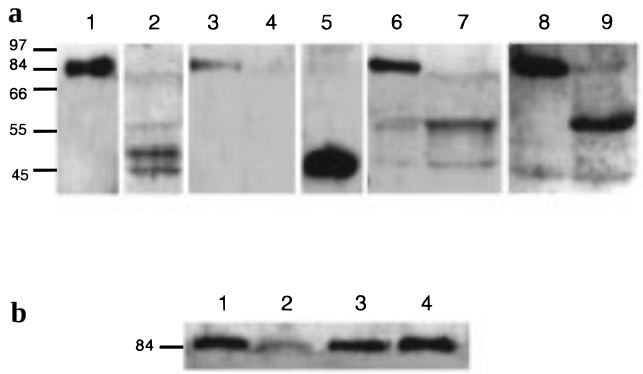

FIGURE 2: Immunoblotting of BBMV, reconstituted BBMV, and differentiated Caco-2 cells. (a) Lane 1, BBMV; lane 2, basolateral membrane preparation from rabbit enterocytes partially purified according to the methods described in ref *45*; lane 3, BBMV treated in the same way as it was for the digestion with *N*-glycanase except that the enzyme was not added; lane 4, BBMV after deglycosylation; lane 5, purified Lum CLA-1; lanes 6 and 8, differentiated Caco-2 cells treated as it was for the digestion with *N*-glycanase except that the enzyme was left out; and lanes 7 and 9, differentiated Caco-2 cells after deglycosylation. In lanes 1−7, affinity-purified pAb 1336 IgG was the primary antibody, whereas in lanes 8 and 9, pAbI2 IgG was used. (b) Lane 1, BBMV; lane 2, fraction of BBMV proteins that was insoluble in 30 mM diheptanoyl PC as described in ref *8*; lane 3, fraction of BBMV proteins solubilized with 30 mM diheptanoyl PC (*8*); and lane 4, BBMV proteins reconstituted to an artificial membrane system (proteoliposomes) according to the methods described in ref *8*. The primary antibody used was affinity-purified pAb 1336 IgG. In each lane of panels a and b, 50 μg of protein was applied except for lane 5, in which 25 ng of Lum CLA-1 was loaded. The position of molecular mass markers in kilodaltons is given on the left.

band (Figure 2a, lanes 1 and 6) with an apparent molecular mass of ∼84 kDa, consistent with the value reported for SR-BI (*23*, *29*, *30*). In contrast, this band was absent in immunoblots of basolateral membrane vesicles prepared from rabbit enterocytes (Figure 2a, lane 2). Immunoblot analysis of BBMV and Caco-2 cells using anti-human SR-BI IgG raised against the C-terminal peptide of the SR-BI isoform (pAbI2) gave a major band at 84 kDa with Caco-2 cells (lane 8) but not with BBMV (not shown). The postnuclear lysate of mouse Y1-BS1 adrenal gland cells known to contain SR-BI was used as a positive control. It yielded a single band in exactly the same position as the bands observed with BBMV and Caco-2 cells. When the positive control was treated with *N*-glycanase, the band at 84 kDa was replaced by a 57 kDa band (see below). If the anti-human SR-BI antiserum or IgG was incubated with purified Lum CLA-1 protein prior to immunoblotting, the 84 kDa band was absent. Also, immunoblotting of BBMV and Caco-2 cells using preimmune serum or IgG gave no band at 84 kDa (data not shown). When BBMV and Caco-2 cells were deglycosylated prior to immunoblotting, the intensity of the 84 kDa band was markedly reduced or disappeared, and a new band at ∼57 kDa appeared (Figure 2a, lanes 4, 7, and 9; in the reproduction of Figure 2a, the 57 kDa band in lane 4 is not visible but it could clearly be detected by eye in the original). Thus, BBMV and Caco-2 cells gave the same result as the positive control, and this result is entirely consistent with the *N*-glycanase digestion of SR-BI reported in the literature (*23*, *31*). The molecular mass of the new band of 57 kDa is in excellent agreement with the value calculated from the amino acid sequence of human SR-BI.

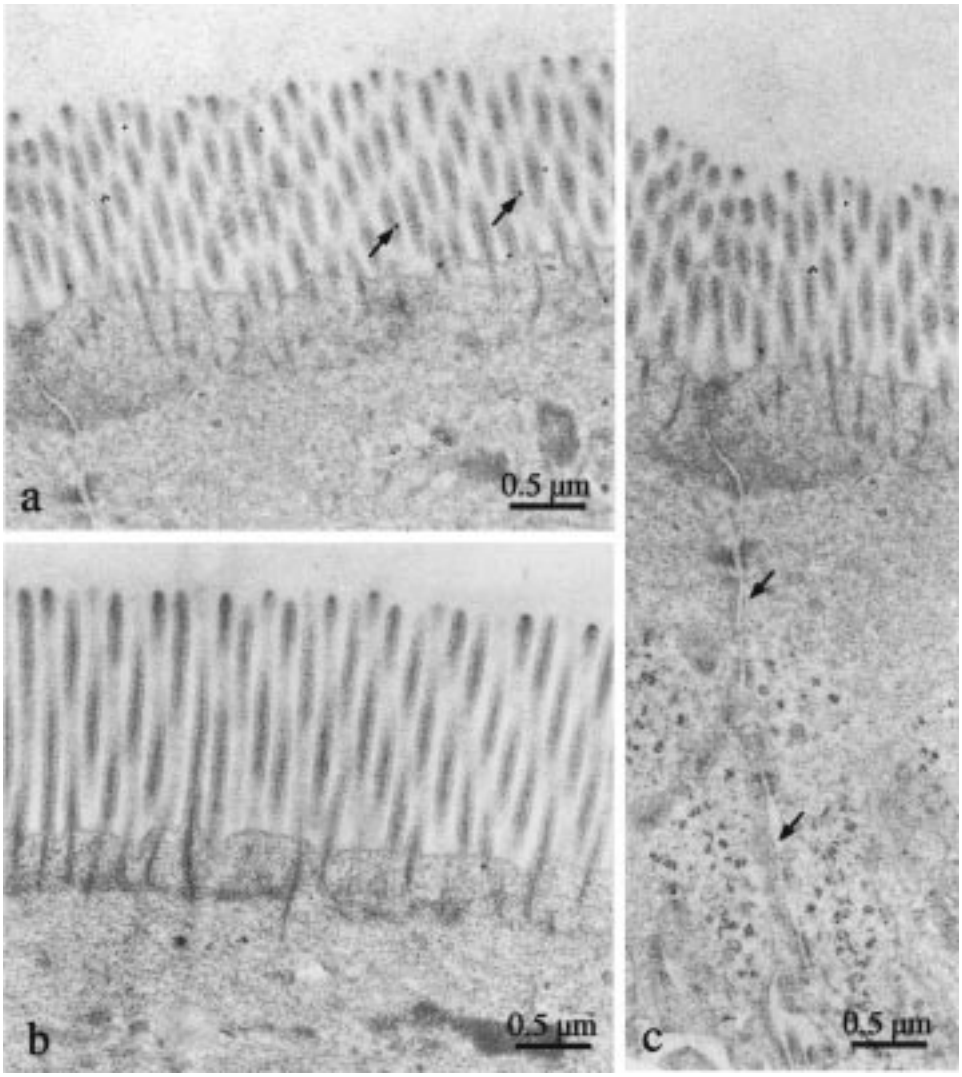

FIGURE 3: Immunoelectron microscopy. Localization of SR-BI in the rabbit BBM by immuno-gold labeling. (a) Rabbit small-intestine section prepared and treated with rabbit anti-human SR-BI IgG and protein A-15 nm gold as described in ref *19* (arrows). (b) Control, a section similar to panel a treated with preimmune serum and protein A-15 nm gold. (c) Section treated as described for panel a showing the basolateral membrane (arrows).

When rabbit BBMV were solubilized in excess detergent (diheptanoyl PC), the sterol transport protein was present in the soluble fraction but not in the detergent-insoluble fraction, as is evident from lipid uptake measurements reported previously (*8*). Immunoblotting of the soluble and insoluble fractions (Figure 2b, lanes 3 and 2, respectively) showed that SR-BI is present mainly in the soluble fraction. The sterol transport activity could be reconstituted into large unilamellar vesicles (proteoliposomes) (*8*), and immunoblotting of these vesicles yielded a single band at 84 kDa (Figure 2b, lane 4). The results of Figure 2 indicate that the sterol transport activity is associated with SR-BI, an integral BBM protein that can be reconstituted into phospholipid bilayers.

The results of the immunoblot analyses were confirmed by immunoelectron microscopy. Immuno-gold labeling of rabbit small-intestine sections using rabbit anti-human SR-BI (pAb 1336) IgG revealed the presence of SR-BI in the BBM but not in the basolateral membrane (Figure 3). The extent of gold labeling of the BBM produced by anti-human SR-BI is relatively weak compared to that produced by antibodies raised against sucrase−isomaltase or lactase phlorizin hydrolase, two of the most abundant proteins of the BBM (data not shown). Nevertheless, statistical analysis of electron micrographs analogous to that shown in Figure 3 indicated that the gold labeling produced by anti-human SR-BI IgG is significant. The density of the gold labeling of the BBM domain produced by anti-human SR-BI IgG was 630 ± 200 gold particles/mm^2 tissue section; using pre-immune IgG, this value dropped to an insignificant level of 50 ± 50 particles/mm^2 characteristic of background labeling.

To demonstrate that SR-BI present in the BBM is active in facilitating lipid uptake, the experiments summarized in Figures 4 and 5 were performed. Figure 4a shows that the uptake of cholesteryl oleate from egg phosphatidylcholine (PC) SUV into BBMV is protein-facilitated and exhibits biphasic kinetics. The half-times for the first and the second kinetic phase derived from curve fitting were 1.7 ± 0.4 min and 2.1 ± 0.4 h, respectively. Anti-human SR-BI (pAb 1336) IgG partially inhibited the uptake of cholesteryl ester, and the value for half-maximum inhibition was 0.29 ± 0.01 mg of IgG/mL. In the presence of the antibody, the size of the initial fast phase of cholesteryl oleate uptake was reduced maximally to $52 \pm 2\%$ (Figure 4b), whereas the same amount of preimmune IgG had no effect on cholesteryl ester uptake.

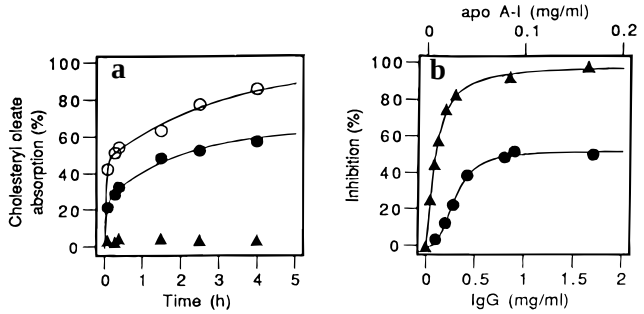

FIGURE 4: (a) Kinetics of cholesteryl ester uptake by rabbit BBMV. SUV of egg PC containing 1 mol % cholesteryl oleate labeled with [^{3}H]cholesteryl oleyl ether (0.05 mg of total lipid/mL) were incubated at 23 °C with BBMV (5 mg of protein/mL and 3 mg of total lipid/mL) in the absence (○) and presence of either 1.4 mg/mL anti-human SR-BI IgG (●) or 0.14 mg/mL human apo A-I (▲). Cholesteryl oleyl ether was used as a lipase-resistant analogue of cholesteryl oleate, and it was shown previously (*6*) that the uptake kinetics of cholesteryl oleate and cholesteryl oleyl ether are identical. The solid lines were obtained by fitting the experimental points (average ± SD, $n = 3$) by a double-exponential equation (*6*, *7*). (b) Percent inhibition of cholesteryl oleate uptake by BBMV as a function of increasing quantities of either apo A-I (▲) or anti-human SR-BI IgG (●). Dose−response curves were constructed from rates of lipid uptake as described in ref *15*. Rates of cholesteryl ester uptake were calculated from data points (average ± SD, $n = 3$) obtained after incubation for 20 min (cf. panel a). The solid lines were obtained by curve fitting using a modified Hill equation as described previously (*15*). In both panels a and b, the standard deviation was less than 5%, and the error bars were smaller than the size of the symbols and therefore omitted.

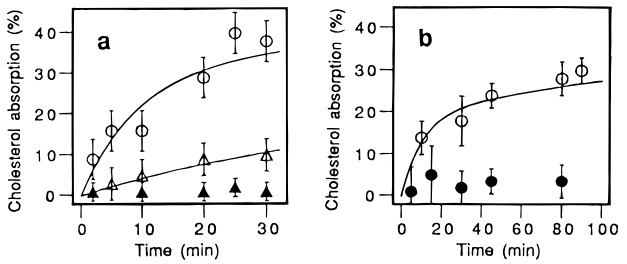

FIGURE 5: Cholesterol absorption by Caco-2 cells measured at 37 °C in the absence and presence of HDL-related inhibitors and anti-human SR-BI (pAb 1336) IgG. (a) Cholesterol absorption from mixed bile salt micelles [4.8 mM sodium taurocholate, 0.6 mM sodium oleate, and 4 μM (0.07 mol %) [^{3}H]cholesterol] in the absence of inhibitors (○) and in the presence of either $18A_D$ (0.3 mg of protein/mL) (△) or HDL reconstituted from human apo A-I (0.2 mg of protein/mL) and dimyristoyl PC (▲) according to the methods described in ref *15*. The solid lines represent fits to the experimental data (average ± SD, $n = 3$) using a double-exponential function. (b) Cholesterol absorption from SUV of egg PC/egg phosphatidic acid (1:1 mole ratio) containing 1 mol % [^{3}H]-cholesterol at 0.05 mg of total lipid/mL in the absence (○) and presence (●) of anti-human SR-BI (pAb 1336) IgG (1.15 mg of protein/mL). The solid line represents a fit to the experimental data (average ± SD, $n = 3$) using a double-exponential function.

Apo A-I was shown to be a ligand for SR-BI (*11*, *32*). In the presence of apo A-I at 0.14 mg/mL, the uptake of cholesteryl ester was completely inhibited (panels a and b of Figure 4). The kinetics of cholesterol uptake from SUV were similar to those for cholesteryl ester uptake, and both apo A-I and anti-human SR-BI IgG inhibited the uptake in a manner similar to the results shown in Figure 4.

The data presented above show that SR-BI is present in the BBM and facilitates the uptake of both free and esterified cholesterol into isolated BBMV. As pointed out by our laboratory previously (*7*, *18*), it is important to verify results obtained with BBMV in other BBM models more closely related to the in vivo situation. The differentiated Caco-2 cell represents a reasonable model of the BBM functional in lipid uptake (*18*). In Figure 5, the kinetics of cholesterol uptake by Caco-2 cells are presented in the absence and presence of HDL-related inhibitors and anti-human SR-BI (pAb 1336) IgG. In the presence of reconstituted HDL (0.2 mg of protein/mL), cholesterol absorption was effectively inhibited (Figure 5a), and this was also the case in the presence of human SR-BI IgG (1.15 mg/mL) (Figure 5b). Apo A-I was found to be totally or partially degraded by proteases released from the cells, and its inhibitory effect on sterol uptake was correspondingly reduced (data not shown). Therefore, the peptide $18A_D$ was synthesized from D-amino acids to make it resistant to proteolysis and degradation during incubation with Caco-2 cells. This peptide was shown to interact analogously to apo A-I (*15*) and at 0.3 mg/mL reduced the initial rate of cholesterol absorption by Caco-2 cells about 4-fold (Figure 5a). The experiments with Caco-2 cells (Figure 5) confirm the results obtained with BBMV and provide clear-cut evidence for SR-BI being functional in an intact cell system. Lipidated apo A-I and $18A_D$ that appear to be specific ligands for SR-BI effectively inhibited cholesterol absorption in Caco-2 cells. It should be noted that free cholesterol taken up by the Caco-2 cell is internalized and metabolized. As a result, radioactive free and esterified cholesterol appear in the cell, and furthermore, radiolabeled sterols are secreted into the basolateral compartment (*18*).

Table 1: Uptake of Various Lipids by Brush Border Membrane Vesicles

lipid	lipid uptake[a] (pmol/mg of BBM protein) native BBMV (control)	proteolytically treated BBMV[b]	native BBMV and apo A-I[c]
cholesterol	98 ± 12	10 ± 5	45 ± 2
cholesteryl oleate	96 ± 5	not detectable	48 ± 5
trioleoylglycerol	127 ± 20	not detectable	51 ± 8
dipalmitoyl PC	127 ± 13	not detectable	48 ± 6

[a] Rates of lipid uptake (mean ± SD of three to five measurements) were determined at 23 °C over a period of 20 min. Egg PC donor SUV containing 1 mol % labeled lipid at 0.2 mg of total lipid/mL were incubated with rabbit BBMV at 4.2 mg of protein/mL in phosphate-buffered saline [0.01 M sodium phosphate (pH 7.4), 0.12 M NaCl, and 2.7 mM KCl]. [b] Rates of lipid uptake measured with BBMV that were pretreated with proteinase K or papain (*14*) to remove SR-BI responsible for facilitated lipid uptake. [c] Rates of lipid uptake measured with native BBMV in the presence of 10 μg/mL human apo A-I as an inhibitor (cf. Figure 4).

An important property of the lipid or scavenger receptor of the BBM is evident from the data compiled in Table 1. For BBMV, similar rates of lipid uptake within experimental error were measured for free and esterified cholesterol, triacylglycerol, and dipalmitoyl PC. After proteolytic treatment of BBMV with proteinase K or papain (*14*), the uptake of hydrophobic, water-insoluble lipid molecules such as cholesteryl ester, trioleoylglycerol, and dipalmitoyl PC was no longer measurable. In contrast, the residual rate of cholesterol uptake was 10 pmol/mg of BBM protein or 10% of that measured with native BBMV. This rate is consistent with simple passive diffusion of cholesterol between two

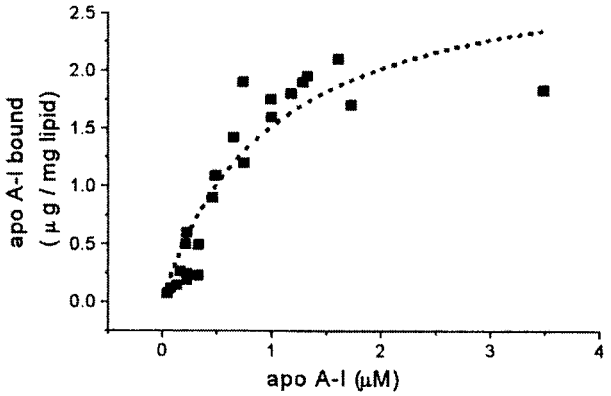

FIGURE 6: Binding isotherm for the interaction of apo A-I with BBMV. The experimental conditions are given in Methods. The dotted line was obtained by curve fitting using the following form of the Scatchard equation, $c_b/L = n(c_w/K_d)/(1 + c_w/K_d)$, where c_w and c_b are the concentrations of free and bound apo A-I, respectively, L is the total lipid concentration of BBMV, n is the number of apo A-I binding sites per membrane lipid molecule, and K_d is the dissociation constant.

populations of SUV (*5*−*7*). As is evident from Table 1, apo A-I at 10 μg of protein/mL inhibited the uptake of all four classes of lipids to the same extent, i.e., by about 50%.

The binding isotherm for the interaction of apo A-I with BBMV at room temperature is shown in Figure 6. The values for K_d and n derived from curve fitting were 1.0 ± 0.1 μM (30 ± 5 μg/mL) and 3.0 ± 0.5 μg of apo A-I/mg of BBM lipid (1.5 ± 0.2 μg of apo A-I/mg of BBM protein), respectively.

DISCUSSION

The finding that anti-human SR-BI IgG inhibits sterol uptake in both BBMV and Caco-2 cells (Figures 4 and 5) is taken as direct evidence for SR-BI being the lipid receptor of the small-intestine BBM that is responsible for the facilitated uptake of sterols and other lipids (Table 1). While anti-human SR-BI IgG abolished the protein-mediated cholesterol uptake in Caco-2 cells (Figure 5b), the maximum inhibition observed with BBMV was 50% (Figure 4). The difference may be due to the limited cross-reactivity of anti-human SR-BI IgG with SR-BI of rabbit BBMV. Together, the immunoblot (Figure 2) and the immunoelectron microscopy evidence (Figure 3), the kinetic data (Figures 4 and 5), and the binding studies (Figure 6) led us to conclude that the lipid uptake activity of the small-intestine BBM is associated with SR-BI. Immunoblotting and immunoelectron microscopy provide good evidence that SR-BI is located in the BBM of enterocytes but not in the basolateral membrane (Figure 2a, lanes 1 and 2). It is expected that SR-BI as an integral membrane protein can be solubilized from BBMV in excess detergent and reconstituted into lipid bilayers. This expectation is borne out by experiment; the reconstituted membrane vesicles closely resembled the original BBMV in terms of lipid uptake behavior (*8*), and immunoblotting of the reconstituted membranes confirmed the presence of SR-BI (Figure 2b, lane 4). Northern blot analysis revealed that the message of SR-BI is present in human enterocytes and Caco-2 cells at levels comparable to that of human liver.

Data in the literature lend further support to the notion that SR-BI is the lipid receptor of the BBM responsible for facilitated lipid uptake. For instance, the binding of HDL to membrane-resident SR-BI (*30*, *32*) has been shown to mediate transfer of free and esterified cholesterol from the HDL particle to the cell membrane, and this transfer was shown to be inhibited in the presence of anti-SR-BI antibodies (*17*). This kind of sterol transfer referred to as selective sterol uptake plays an important role in the receptor-mediated delivery of sterols to steroidogenic tissue such as the adrenal gland (*33*). It is important to note that lipid uptake by the BBM from lipid donor particles closely resembles the selective sterol uptake. In both cases, the mechanism, though not well understood, is certainly distinct from receptor-mediated endocytosis. Our kinetic data are consistent with collision-induced lipid transfer from the donor particle to the acceptor membrane, and this transfer is catalyzed by SR-BI. Recent studies have provided compelling evidence that SR-BI also participates in the movement of cholesterol from cell membranes to HDL, a process referred to as reverse cholesterol transport (*34*−*36*). Furthermore, transfection of cells with either human or murine SR-BI increases both the extent of selective uptake (*30*, *37*) and the reverse sterol transport (*35*). Thus, this receptor can facilitate the bidirectional flux of lipid molecules between the bound ligand and the cell membrane. This is entirely consistent with lipid uptake measurements; work from our laboratory (*14*, *38*) provided convincing evidence for the bidirectional lipid flux between donor particles and the acceptor membranes (BBMV).

HDL and apo A-I have been shown to be physiological ligands of SR-BI (*11*, *23*, *30*, *32*, *37*). The binding of apo A-I to BBMV is characterized by a dissociation constant K_d of 1.0 ± 0.1 μM and a value for maximal binding n of 1.5 ± 0.2 μg of apo A-I/mg of BBM protein (Figure 6). These values are in good agreement with data for the binding of HDL to the plasma membrane of cells transfected with either human or murine SR BI (*30*, *37*). The good agreement points to SR-BI of the BBM being responsible for the binding of apo A-I and HDL. Evidence presented in Figures 4 and 5 and Table 1 indicates that binding of apo A-I or HDL to SR-BI inhibits sterol and lipid uptake with an IC_{50} value of 9.2 ± 0.3 μg/mL. The L-amino acid homologue of $18A_D$ was previously shown to inhibit the uptake of free and esterified cholesterol into BBMV (*15*). This peptide is an effective inhibitor because it is amphipathic, has the propensity of forming an α-helix of type A, and thus mimics the interaction of apo A-I with SR-BI. The observation that the inhibition of sterol uptake by HDL and apo A-I reaches a level of 100% in both BBMV and Caco-2 cells (Figures 4 and 5) is interpreted to indicate that only receptors for apo A-I are involved in facilitating sterol uptake. Although this result supports a major role of SR-BI in sterol absorption, a contribution from other receptors cannot be ruled out. Likely candidates are other scavenger receptors, isoforms of SR-BI and CD36, an SR-BI-related molecule which was shown to bind HDL (*39*) and at least in rat intestine to be present in the microvilli of enterocytes (*40*). The data presented in Figures 4 and 5 also demonstrate that SR-BI facilitates sterol uptake from different lipid donor particles, including neutral and negatively charged SUV and bile salt micelles. This finding is consistent with published data showing that SR-BI is a multiligand receptor that, in addition to native and modified lipoproteins and apoproteins, binds anionic lipids (*11*, *23*).

From the data presented in Table 1, the following conclusions can be drawn. (I) The uptake of water-insoluble lipids into the BBM is protein-facilitated. (II) The fact that apo A-I inhibits the uptake of all four lipids with identical IC_{50} values is interpreted to mean that SR-BI is responsible for the facilitated uptake of not only sterols but also other lipids such as triacylglycerols and phospholipids. Further, the data of Table 1 allow us to assess the relative contributions of simple, passive diffusion and facilitated diffusion to lipid uptake. Protease treatment of BBMV liberates a significant proportion of proteins from the external surface of the BBM, and in turn the protein-facilitated lipid uptake is lost. As is evident from Table 1, after digestion of BBMV with proteinase K or papain, the contribution of simple passive diffusion to the uptake of hydrophobic lipids such as cholesteryl oleate, trioleoylglycerol, and dipalmitoyl PC was negligible, i.e., too small to be measurable. Apparently, uptake of these lipid molecules occurs solely by the facilitated pathway. With less hydrophobic lipid molecules such as cholesterol, about 10% of the total cholesterol uptake is due to simple passive diffusion (Table 1). Lipolysis of substrates such as triacylglycerols and cholesteryl esters generates more polar lipid molecules such as free fatty acids and monoacylglycerols, and thereby, lipid uptake by simple diffusion may be promoted (*1*). More work is required to answer the question of whether and to what extent uptake of long-chain fatty acids, monoacylglycerols, and nonpolar vitamins is facilitated by SR-BI or any other lipid transporter of the BBM.

Physiological Significance. SR-BI is the first receptor of the BBM shown to be involved in the absorption of dietary lipids. This discovery substantiates our hypothesis that intestinal absorption of sterols and other lipids is protein-mediated, a hypothesis primarily based on kinetic analyses of lipid uptake measurements (*5*−*8*). It is apparent from the work presented that for lipid molecules with hydrophobicities similar to or greater than that of cholesterol the pathway of facilitated diffusion predominates over that of simple passive diffusion. SR-BI is of pivotal importance in cholesterol metabolism and homeostasis; it has been shown to play a key role in the transport of free and esterified cholesterol from HDL to cells (selective lipid uptake) as well as the reverse process (reverse cholesterol transport). Results presented here add significantly to our understanding of the physiological function of SR-BI and point to a much broader role for this receptor in cholesterol metabolism; in addition to regulating HDL cholesterol, it affects whole-body cholesterol levels by facilitating absorption of cholesterol in the small intestine. SR-BI exhibits little lipid specificity; it rather functions as a port or "docking receptor". When lipoprotein or lipid particles dock at SR-BI, this receptor mediates the bidirectional flux of lipid molecules almost indiscriminately between the lipid particle and the plasma membrane. Net mass transfer of lipid by this facilitated diffusion process occurs when there is a concentration gradient between the docked particle and the plasma membrane. SR-BI seems to be able to cope with a broad range of hydrophobic molecules which may greatly differ in chemical structure. It is an integral, highly glycosylated membrane protein with most of its mass being exposed on the luminal side of the BBM. The existence of such a receptor in the BBM and possible isoforms differing in transport activity could account, at least in part, for the large interindividual variability in cholesterol absorption (*41*−*43*). Consistent with this idea, it has been shown that the variability in intestinal sterol absorption is one of two possible metabolic causes affecting the low-density lipoprotein cholesterol level in monkeys during dietary cholesterol challenge (*44*). Future studies will address the question of whether structural variations in scavenger receptor and/or other reasons underlie the interindividual variations in cholesterol absorption.

The identification of a scavenger receptor in the intestinal BBM raises the intriguing possibility of manipulating the absorption of dietary cholesterol and triacylglycerols in the intestine. A prerequisite for this approach would be a systematic search for inhibitors of SR-BI and the effective screening of possible inhibitors with a suitable BBM model. Such an approach holds out a prospect of controlling, at the level of intestinal lipid absorption, hypercholesterolemia and obesity, two risk factors of atherosclerosis.

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